J. Glob. Innov. Agric. Soc. Sci., 2014, 2(2): 93-96.
ISSN (Online): 2311-3839; ISSN (Print): 2312-5225
DOI: 10.17957/JGIASS/2.2.549
http://www.jgiass.com

BACTERIOLOGICAL EXAMINATION OF SOME READY-TO-EAT FOODS IN FAISALABAD

Iftikhar Hussain* and Ayesha Sarwar

Institute of Microbiology, University of Agriculture, Faisalabad, 38040-Pakistan
***Corresponding author's e-mail: driftikharuaf@gmail.com**

The aim of this study was to find out bacteriological quality of ready-to-eat (RTE) foods in Faisalabad by ascertaining the bacteria contaminating various RTE foods through Aerobic Plate Count (APC) on nutrient agar and to count the different types of bacteria through APC on selective media for Staphylococci, *E. coli* and Salmonella. A total of 120 samples Dahee baray, goal gappay, fruit chat and patties were collected from different streets and school canteens in Faisalabad city between March and August, 2010. The most prevalent isolated bacteria from Dahee baray, goal gappay, fruit chat and patties were; *Escherichia coli*, *Staphylococcus aureus* and Salmonells sp. The viable bacterial counts were $7.4x10^4$ cfu/ml, 3×10^4 cfu/ml, 2.5×10^4 cfu/ml and 4×10^4 cfu/ml for Dahee baray, goal gappay, fruit chat and patties, respectively.
Keywords: bacteria, Staphylococci, goal gappay, Dahee baray, milk

INTRODUCTION

Ready-to-eat (RTE) foods are describes as the foods being ready for immediate consumption at the point of sale. RTE foods could be raw or cooked, hot or chilled and can b consumed without further heat treatment (Tsang, 2002). Different terms have been used to describe such foods; these include convenient, ready, instant and fast foods e.g., pastries, sausages, rolls, burgers, salad, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974).
Increased consumption of RTE foods result in food-borne illness (Sivapalasingam *et al.*, 2004). Microbiological studies carried out on RTE foods in several developing countries have reported high bacterial counts (Bryan *et al*., 1997, Umoh and Odoba, 1999). Food-borne bacterial pathogens commonly detected in RTE foods were *E. coli* O157:H7, Salmonella species, *Listeria monocytogents*, Campylobacter, *Clostridium perfringens*, *Bacillus cerus*, *Staphylococcus aureus* (Mosupye and Von Holy, 1999). RTE foods have also been implicated in outbreaks of food-borne diseases. In 1981 a cholera epidemic in Pune city, India was attributed to contaminated sugar juice with added ice (WHO, 1996). In Senegal more than 200 cases of food poisoning were reported by RTE foods prepared by dairy products (Dawson and Canet, 1991).
Between 1992-1997 in England and Wales, there were 20 reported outbreaks of food poisoning are attributed to consumption of contaminated cold meat from catering premises with 573 people affected in months of June to August. Cold meat consumption resulted in salmonella outbreak (Little *et al*., 2000). Microbiological quality of fruit chat assessed in Patiala city of India showed food borne illness in consumers (WHO, 1998). Standard techniques have been used to access the microbiological quality of RTE foods including spread plate technique which separates duplicate APC and Gram staining (GS) (Mosupye and Von Holy, 1999). The consumption of RTE foods has increased dramatically in recent years. It is important to know the type and number of different bacteria contaminating the RTE foods.
The study was designed to isolate, count and differentiate the bacteria contaminating various RTE foods through APC on nutrient agar and on selective media.

MATERIALS AND METHODS

Samples: A total 120 sample were obtained for bacteriological examination. Various types of RTE foods were collected from different streets and school canteens in Faisalabad form March to August, 2010. Among the 120 samples there were 30 samples of Dahee baray, 30 samples of goal gappay, 30 samples of fruit chat and 30 were of patties. The 120 samples which collected from different streets and school canteens were also grouped on basis of their major component from which these were made *i.e.* 30 samples were mainly composed of milk and milk products, 30 samples were mainly composed of meat and meat products and 30 samples were mainly composed of salads and vegetables.

Samples preparation: All the samples were shifted to the laboratory at room temperature. They were analyzed within 24 hr of sampling. Ten grams of each sample was homogenized with 5 ml normal saline (NS). Serial dilutions were performed as required. Standardize the dilution by selecting that plate which gave dilution within range of 30-300 colonies. That was dilution fourth (4^{th} dilution).

Aerobic Plate Count: After homogenization, the sample for aerobic plate count was serially diluted in normal saline to 10-3, the 10-1 to 10-4 dilutions were plated on plate count agar (Oxide, Basingstoke UK). The plates were incubated at 37C for 24-48 h. counts were made using manufacture instructions and reported on colony forming (cfu/g).

Isolation and identification of E. coli: After homogenization, the sample was serially diluted in NS to 10^{-4}. Using a sterile pipette, 0.5 ml of 10^{-4} dilution was spread on Nutrient agar (Oxide, Basingstoke, UK). The plates were then allowed to dry before being incubated at37^{o}C for 18-24 h. the off white colored colonies were identify as presumptive *E. colli* and confirmed using MacConkey's agar (Oxide, Basingstoke, UK).

Isolation and identification of Salmonella: The homogenate in NS was incubated for 18-24h at 37C followed by selective enrichment of 1 ml in 9 ml of Selenite Cystine broth (SC) or tetrathionate broth. The tetrathionate/SC broth was incubated at 37^{o}C for 18-24h. The broths were then sub cultured onto Salmonells-Sheigella agar (S.S. agar) and incubated at 37^{o}C for 24-48 h (Oxide, Basingstoke, UK). Presumptive positive colonies (non-lactose fermenting with suitable colony morphology) were then confirmed by using IMVIC and Triple Sugar Iron (TSI) (API, Biomerieux, Marcy L'Etoile, and France).

Isolation and identification of Staphyylococcus aureus: Using a sterile pipette 0.5ml of the 10^{-4} dilution was spread onto a plate of Staph. 110 Agar (Oxoid, Basingstoke, UK). The plates were then allowed to dry before being incubated at 37^{o}C for 48 h. The plates were then examined for typical *Staph. aureus* colonies (golden yellow, shiny, convex colonies with a narrow zone of opacity surrounded by a zone of clearing). The presence of *Staph. aureus* was confirmed by the catalase and coagulase tests (Pro-Lab Prolex Staph kit, Neston, Wirral, UK).

Statistical analysis: The data is analyzing through Chi-Square test. The correlation between variety of food and bacterial load is less than 5.

RESULTS

The microbial analysis of some RTE foods is presented in table 1, 2 and 3. Regarding the distribution of microbial population highest bacterial load observed in Dahee baray, which is (51.25%) for *E. coli*, followed by Salmonella, which is (33.3%), and then *Staph. aureus* is (16.67%). The percentage of *E. coli* in Dahee baray is (50%) followed by Salmonella (33.3%) and *Staph. aureus* is (16.67%). The percentage of *E. coli* in fruit chat is (46.67%) followed by Salmonella (33.3%) then *Staph. aureus* is (20%). The bacterial load in Patties was (56.67%) in *E. coli* followed by (30%) Salmonella and (13.3%) *Staph. aureus*.

DISCUSSION

Ready-to-fast foods (RTE) are described as the foods being ready for immediate consumption at the point of sale. RTE foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsng, 2002). Different terms have been used to describe such foods; these include convenient, ready, instant and fast foods *e.g.*, pastries, sausages, rolls, burgers, salad, fried meat, fried chicken, milk and milk products (Caserani and Kinston, 1974). RTE foods are prepared in the streets for immediate consumption or for consumption without further processing (Dawson and Canet, 1999).

120 samples were randomly selected from different streets and schools canteens around Faisalabad. The samples were subjected to bacteriological examination to check the presence of *E. coli*, Salmonella and *Staph. aureus*. From first type of meat and meat products the major pathogen reported were *E. coli* (6.24%)/g, Salmonella (4.5%)/g and Staph. Aureus (1.37%)/g of patties which was isolated from raw beef patties where *E. coli* was (92%)/g, *Staph. aures* (82%)/g followed by Salmonella 0.4%/g of the sample (Barnard et al., 1975)

Previously work was done for isolation of bacteria from ready-to-eat food in Faculty of Veterinary Sciences, Instiitute of Microbiology, UA Faisalabad in 2003 but the pathogen isolated were *L. monocytogenes* and Compylobacter. There are various typea of ready to eat foods like milk and milk products, meat and meeat products, salads and vegetables and juices etc. From the first category

Table 1: Aerobic Plate Count (APC) of different Bacteria on General and Selective media

Control	Sr. No	Isolates	APC on Nutrient agar (cfu/ml)	APC on MacConkey agar (cfu/ml)	APC on S.S agar (efu/ml)	APC on Staph. 110 agar (efu/ml)
Fresh food	1	D1	$2.1x10^5$	$7x10^4$	$2x10^4$	$3x10^4$
	2	D2	$2.3x10^5$	$7.4x10^4$	$3x10^4$	--
	3	P1	$2.4x10^5$	$1.1x10^3$	$4x10^4$	$4x10^4$
	4	P2	$2.9x10^5$	$2.5x10^3$	--	$3.8x10^4$
	5	G1	$3x10^5$	$2.7x10^3$	$4x10^4$	$5x10^4$
	6	G2	$3.5x10^5$	$2.5x10^5$	$3x10^4$	$4x10^4$
	7	C1	$2.7x10^5$	$1.7x10^3$	$6x10^4$	$5x10^4$
	8	C2	$2.9x10^5$	$2x10^3$	$5x10^4$	$3.5x10^4$
Refrigerated food for two days	1	D1	$2.2x10^5$	$6.9x10^4$	$2x10^4$	$1x10^3$
	2	D2	$2.4x10^5$	$7.1x10^4$	$2.9x10^4$	$2.5x10^3$
	3	P1	$2.6x10^5$	$1x10^3$	$3.9x10^3$	$3.1x10^4$
	4	P2	$2.9x10^5$	$2.5x10^3$	--	$4x10^4$
	5	G1	$3.1x10^5$	$1.2x10^3$	$2.5x10^5$	$3x10^4$
	6	G2	$3x10^5$	$2.4x10^3$	$2.7x10^4$	$4x10^4$
	7	C1	$2.3x10^5$	$1.1x10^5$	$1.3x10^3$	$4x10^4$
	8	C2	$2.5x10^5$	$1.8x10^3$	$4x10^4$	$4x10^4$

Table 2: Percentage (%) of bacterial isolates per ml from RTE foods

Sr. No	RTE foods	*E. coli*	Salmonella	*Staph. aureus*
1.	Dahee baray	5.82%	6.41%	1.32%
2.	Goal gappay	5.45%	4.55%	1.32%
3.	Fruit chat	5.95%	5.0%	1.25%
4.	Patties	6.24%	4.5%	1.37%

Table 3: Microbial evaluation and distribution of some RTE food products

Micro-organism	Percentage of Bacteria in some RTE food products			
	Dahee baray	Goal gappay	Fruit chat	Patties
E. coli	15 (50%)	16 (53.3%)	14 (46.67%)	17 (56.67%)
Salmonella	10 (33.3%)	9 (30%)	10 (33.3%)	9 (30%)
Staph. aureus	5 (16.67%)	5 (16.67%)	6 (20%)	4 (13.33%)

of milk and milk products, Dahee baray was selected, from meat and meat products patties selected, from salads and vegetables goal gappay and fruit chat.

Bernard et al, (1972) studied the bacterial counts of samples which was 100/g for *E. coli*, 100/g for *Staph. aureus* and 54/g of Salmonella, which were less than the present study in which *E. coli* count was (6.24%)/g, *Staph. aureus* (4.5%)/g and Salmonella (1.37%)/g. the present study correlates with past studies on the bacterial counts and different factors were responsible factors were responsible in increase and decrease count.

Bacterial count is associated with hygienic measures and type of food and utensils used for processing of these RTE foods. The bacterial load due to unhygienic measures, personal bygiene was observed 78% due to *E. coli*, 68% due to Salmonella and 17.5% due to *Staph. aureus*. This study correlates with past studies in which more microbial count was observed which 96.3% was for *E. coli*, 81.5% for Salmonella 71.4% for *Staph. aureus* (Badrie et al., 2003). Sucdari et al. (1996) observed an outbreak due to consumption of iceberg lettuce which comes under category of salads and vegetables and in this study samples chosen was goal gappay and fruit chat. The major bacteria responsible for causing food-borne illness was Salmonella 81% which correlate with present study in which Salmonella isolated from goal gappay was 15.6% which was less than that salmonella isolated from leaf lettuce. In case of milk and milk products sample selected was Ice cream. (Hennessey et al., 1996) observed gastroenteritis due to consumption of Ice cream and pathogen detected was Salmonella (3%). This study correlates with present study in which Dahee baray was selected from category of milk and milk products in which Salmonella count was (6.41%)/g which was more than that isolated from ice cream.

CONCLUSION

It is concluded that that RTE foods are cheap, economical but not healthy due to lack of hygienic measures, dirty utensils, vendor's hygiene etc. these factors contributing many species of bacteria but major pathogen are *E. coli*, Salmonella and *Staph. aureus*. Basic and main source of bacteria infection is poor hygienic measures and this problem may be solved by improving by supervision in food handling procedure, greater consumer education on transmission of enteric food borne diseases and food safety risks.

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